TRANSACTIONS OF THE AMERICAN CLINICAL AND CLIMATOLOGICAL ASSOCIATION, VOL. 112, 2001

# PATHOGENESIS OF CYSTIC FIBROSIS AIRWAYS DISEASE

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## INTRODUCTION

Cystic fibrosis (CF) lung disease is manifested by unrelenting bacterial infections of airway surfaces. Thus, CF reflects a failure of airways defense against inhaled bacterial organisms, more specifically, bacteria of the Staphylococcus and Pseudomonas genuses. Elucidation of the pathogenesis of CF airways disease requires knowledge of the underlying lung defense mechanisms that airways possess against bacterial infections, the functions of cystic fibrosis transmembrane conductance regulator (CFTR) protein in airway epithelial lung defense, and finally, how the genetic mutations associated with the CFTR gene and missing CFTR protein function derange airways defense mechanisms. Precise knowledge of the pathogenesis of CF is required for designing novel and specific therapies to treat the root cause of this disease. Thus, this review is divided into three chapters, focusing on: 1) normal defense of airways against bacterial infection; 2) the effects of missing CFTR function in airways defense; and 3) novel therapeutic strategies for the treatment of CF lung disease.

### Normal Airways Defense:

Surprisingly, in the year 2000, there is no general agreement on the normal mechanisms of airways defense and the role of the airway epithelial cells in this function (1). As depicted in Figure 1, the surface area of the airways expands greatly from proximal to peripheral regions and the relative surface area of the bronchiolar as compared to tracheal regions can be depicted as an "inverted funnel." In the "mechanical clearance" hypothesis of lung defense (depicted on the left), bacteria that impact on airway surfaces are removed via the cephalad clearance of mucus (2). The clearance of mucus from airway surfaces

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Supported by NIH HL34322 and CFF R026

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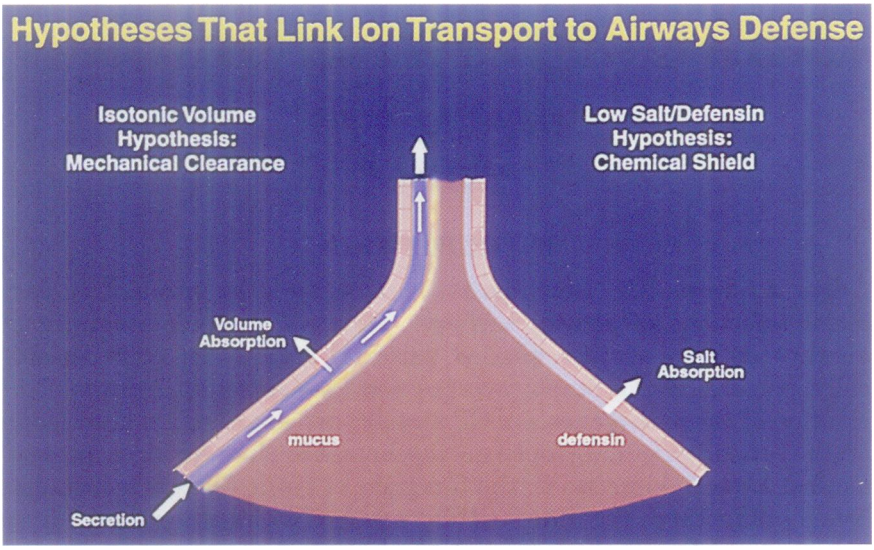


FIG. 1. Depiction of Defense Mechanisms that Defend Airways from Chronic Bacterial Infection. Lung is depicted as inverted funnel, with the mechanical clearance of mucus hypothesis depicted on the left and the chemical shield hypothesis depicted on the right.

requires the highly coordinated activities of the elements that secrete the proper amount of mucins, regulate the quantity of airway surface liquid (salt and water), and coordinate the activity of ciliary beating. Interestingly, the epithelium is not innervated and all of this coordinated activity likely requires autocrine/paracrine regulation of the epithelial cells themselves.

An opposing hypothesis, the "chemical shield" hypothesis for lung defense, is also shown in Fig. 1 (right) (3). According to this hypothesis, bacteria that are inhaled and deposited onto airway surfaces are "removed" from the lung via killing as a result of contact with antimicrobial substances in airway surface liquids (ASL). The central tenet of this hypothesis is that the antimicrobial substances responsible for killing, defensins, are only active in low salt solutions (<50 mM NaCl). Thus, this hypothesis predicts that normal ASL is hypotonic and that hypotonicity serves to "activate" defensin-like molecules in ASL.

Although debate persists, studies have emerged that directly compare predictions derived from these two hypotheses. Perhaps the most direct test of the two hypotheses focuses on the ionic composition of normal ASL. The mechanical clearance hypothesis proposes that the volume of airway surface liquid is critical for effective mucus clearance, and this regulation is effected by the isotonic volume absorptive/secre-

tory capacities of the normal airways epithelium. Thus, the composition of ASL is predicted to be isotonic by the mechanical clearance hypothesis. In contrast, as noted above, the chemical shield hypothesis posits a low salt (hypotonic) ASL. Measurements of ASL are difficult because of the thinness of the layer (depth ~10–30 $\mu$) and the fact that interventions designed to collect airway surface liquids may well perturb the system. Two studies measuring composition of airway surface liquids in cell culture systems, which used isotopic, quick-lavage techniques (4), and one in mice *in vivo* (5), which used a microcapillary collection technique and indirect measurements of ions, have suggested that airway surface liquids are hypotonic. However, the vast majority and most recent measurements indicate that ASL indeed is isotonic. Measurements consistent with isotonic ASL have been made in *in vitro* cell culture systems with ion-selective microelectrodes, fluorescent probes, and microcapillary sampling with direct measurements of ions by using atomic absorption spectroscopy (2). These *in vitro* data are complemented by a large number of studies of ASL collected *in vivo* from a series of large mammals (dogs, sheep, ferrets, pigs) and human upper and lower airways with filter papers and ion-selective microelectrodes (6,7). These data, coupled with the failure to identify salt-sensitive defensins in airway surface liquids in biologically meaningful concentrations, argue strongly for the mechanical clearance hypothesis of lung defense.

**Failure of Airways Defense in Cystic Fibrosis:**

Figure 2 depicts the "microanatomy" of the mucus transport functions of normal airway epithelia and how they are deranged in cystic fibrosis. As shown in the normal state (Figure 2, upper panel), mucin macromolecules are organized into a mucus layer that contains approximately 2% solids (about half of which is the mucin macromolecule content) that overlies an aqueous periciliary liquid layer. The airway surface liquid that is contained in both the periciliary liquid layer (PCL) and the mucin layer is isotonic in salt concentration. The periciliary liquid layer (~7 $\mu$ in depth) is the layer in which the cilia beat in a coordinated fashion at ~20 Hz. The coordinated activities of these three different systems, mucus, ASL, and ciliary beat, move mucus as a sheet from the distal airways to the mouth at approximately 60 $\mu$ per second.

In CF, there is a failure of the airways epithelium to normally regulate ASL volume. The ion transport mechanisms that account for this defect are shown in Figure 3. Under basal conditions, $Na^+$ is

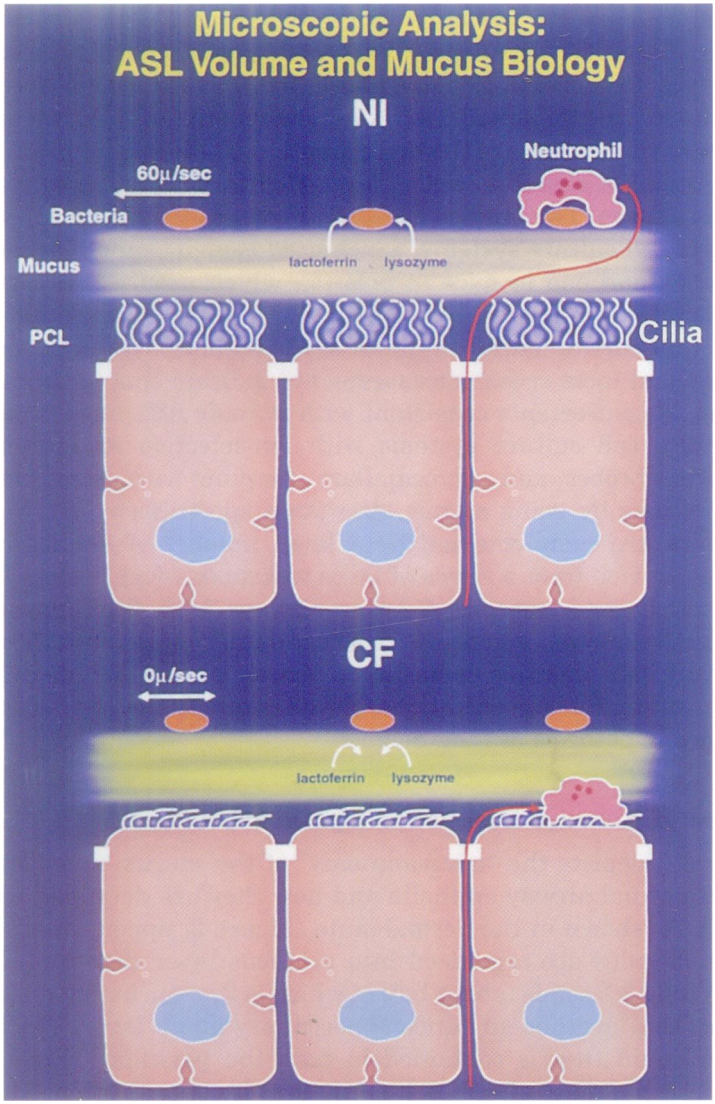


FIG. 2. Depiction of Airways Defense Provided by Mucus Layer. In normal airways (NI - upper panel), mucus traps inhaled bacteria and transport them (vector depicts transport rate of ~60 $\mu$/s) to the mouth for swallowing. "Back-up" mechanisms include local anti-microbial factors (lactoferrin and lysozyme) and migratory cells (neutrophils). In lower panel (CF), the effects of ASL volume (salt and water) depletion in the disease cystic fibrosis are shown. The periciliary liquid layer, in which cilia would normally beat, is depleted and the mucus layer concentrated and adherent to the airway cell surface. As a consequence, mucus is not cleared and the back-up defense mechanisms are also rendered inactive.

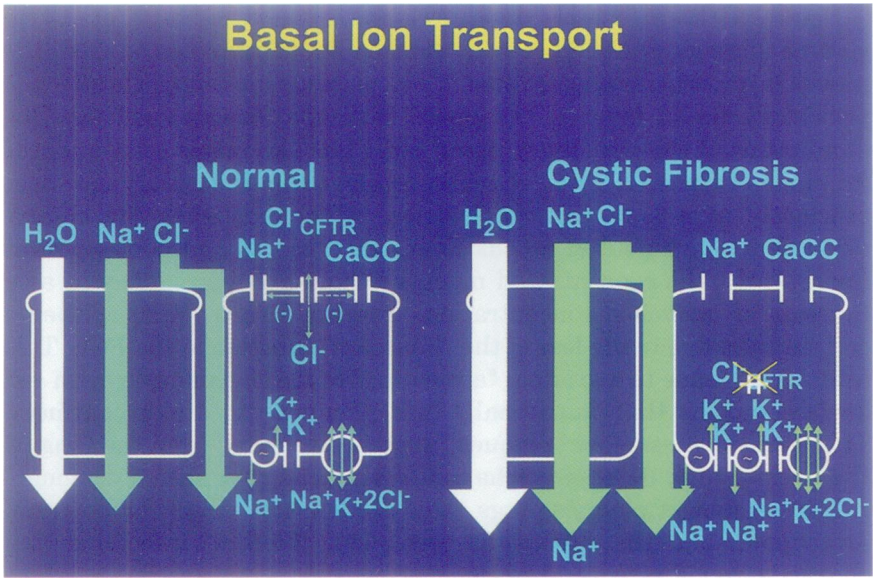


FIG. 3. Depiction of Ion Transport Paths for Normal (L) and CF Airway Epithelia (R). Vectors depict rate and direction of ion transport.

absorbed actively, Cl moves in response to electric gradients across the epithelium as a counterion, and water follows isosmotically across normal airway epithelia (Fig. 3, left). The overall rate of net $Na^+$ (and volume) absorption is set by the activity of the apical membrane $Na^+$ channel, which is regulated in part by CFTR protein. A key concept of the mechanical clearance hypothesis is that the CFTR protein is a central coordinator of volume transport across airways epithelium, with the same protein (CFTR) functioning as both a negative regulator of the epithelial $Na^+$ channel and as a $Cl^-$ ion channel itself. Thus, regulatory influences that, for example, activate CFTR inhibit $Na^+$ absorption and initiate Cl and volume absorption. Thus, the CFTR protein serves to coordinate the requirements of the normal epithelium to regulate the rates of tonic volume absorption and, in certain circumstances, initiate volume secretion to preserve the periciliary liquid layer required for effective mucus transport.

In CF (Figure 3, right), mutations in the CF gene typically lead to a failure to produce normal levels of CFTR protein in cells and target functional CFTR to the apical membrane. The loss of CFTR function at the apical membrane is associated with both a failure to inhibit ENaC, which produces accelerated $Na^+$ absorption, and a limitation on the capacity to initiate Cl secretion. The net effect is that volume absorption is upregulated and cannot be modulated.

Figure 2 (lower panel) illustrates the consequences of excessive volume absorption in CF. Airway surface liquid is extracted from the mucus layer, which concentrates it, but perhaps most importantly, the periciliary liquid layer is "stripped" from the airway surfaces. The elimination of the periciliary liquid layer has two major consequences for mucus clearance. First, the cilia cannot extend normally, nor beat efficiently in a low viscosity solution, which abolishes the ciliary-dependent component of mucus clearance. Second, and perhaps most importantly, the concentrated mucins (which are more adhesive as a function of increased concentration) interact directly with epithelial cell surfaces due to the loss of the "lubricant" function of the PCL. This interaction leads to a gradual "annealing" of the mucus layer with cell surface mucins that functionally "glues" mucus to airway surfaces. These mucus masses, or "plaques," cannot be cleared from the lung by the backup lung defense mechanism for mucus clearance, i.e., cough. Adherent mucus plaques/plugs then become the nidus for bacterial attachment and the surface for persistent "biofilm" infections that characterize CF airways disease.

## Novel Therapies of CF Airways Disease:

The reason it has been so important to differentiate between the two hypotheses that describe normal lung defense is that they predict very different pathogenic mechanisms for the initiation of CF airway disease and hence strategies to treat this disease at its basic cause. The sequence for bacterial infection predicted by the mucus clearance hypothesis is described in the chapter above. The chemical shield hypothesis had predicted that CF airways disease was caused by a failure of the CF epithelium to extract salt from airway surface liquid, with the consequent production of "salty" ASL, inactivation of defensins, and chronic infection. The mechanical clearance hypothesis, which indicates that airway surface liquid volume is depleted in CF, predicts that novel therapies should be aimed at restoring the volume of ASL, which includes strategies to add salt and water back to CF airway surfaces. Conversely, the chemical shield/compositional hypothesis, which suggests that CF airway surface liquids are too salty, predicts that effective therapies should be directed at removing salt from CF airway secretions. It is in part because of these considerations that so much attention has been focused on differentiating between these two competing hypotheses.

With respect to strategies that emanate from the chemical shield/compositional hypothesis, there are no data suggesting that the salt

composition in the ASL covering CF airways is different from normal. Indeed, the only two studies that compared CF ASL prior to infection (in infants) with those of normal subjects detected absolutely no difference in the composition of ASL (8,9). Therefore, to date there have been no serious efforts to develop strategies to remove salt from CF airway secretions. Similarly, there are no data to suggest that there are significant salt-dependent defensins in airway surface liquids (10). Lysozyme and lactiferrin molecules are present in ASL, but their concentrations are so high that they are not salt-sensitive. Despite the absence of evidence for missing defensin-like activity in CF, therapeutic programs have been developed focused on the inhalation of defensin-like molecules as aerosolized antibiotics. However, these classes of molecules appear to be proinflammatory in normal and CF airways and appear to add little to the high defensin concentrations in CF airway secretions that result from lysed neutrophils. Consequently, these trials have not been pursued with vigor.

In contrast, studies designed to restore volume to CF airway surface liquids are evolving. Perhaps the simplest strategy is to have CF subjects inhale hypertonic saline, on the notion that the added saline with draw water rapidly into across the water-permeable airway epithelium. Indeed, there are several studies reporting short-term benefits of hypertonic saline inhalation in CF patients in terms of mucus clearance and lung function over two weeks without any adverse events (particularly increased infection, as would be predicted by the chemical shield/compositional hypothesis) (11,12). Unfortunately, the effects of hypertonic saline are very short-lived, reflecting the capacity of the airway epithelium to absorb the inhaled salt. Consequently, a number of novel drug therapies focused on redressing the imbalance of salt and water on CF airway surfaces are in progress. One class of compounds has capitalized on the discovery that triphosphate nucleotides (UTP) present in the extracellular milieu interact with cell surface 5′ nucleotide receptors (P2Y) that have the capacity to both slow $Na^+$ absorption via inhibition of $Na^+$ channels and initiate $Cl^-$ secretion via an alternative (to CFTR) $Ca^+$-activated $Cl^-$ channel (13). This class of compounds appears to be effective in the acute restoration of volume to CF airway surfaces *in vitro*; stabilized nucleotides have been developed for inhalational use in CF patients and are just beginning Phase I testing.

Another class of compounds that has received considerable attention has been the $Na^+$ channel blockers. The first molecule of this class tested in CF was amiloride. In a variety of acute studies, amiloride increased mucus clearance in normal and CF subjects and perhaps

increased the rate of response to antibiotic therapy in the treatment of acute exacerbations (14). However, the drug in the long term (6 months studies) appeared to exert modest activity by itself and no detectable activity when given in the context of the usual CF therapeutic regimens (15). Reasons for the failure of this compound appear to be pharmacodynamic (a very short duration of action - approximately 1 hr) rather than failure of the concept behind this drug.

## CONCLUSIONS

Thus, there appears to be a number of strategies to effectively increase airway surface liquid volume. The charge to the CF community is to develop drugs that are not only effective but are consistent with chronic usage. Further, it is yet to be known whether, once mucus plaques/plugs become adherent to airway surfaces, restoration of volume is sufficient to reinitiate clearance. A key area of investigation in the future will be whether a second class of drugs will have to be developed that have the capacity to detach these plugs from airway surfaces. It is anticipated that these forms of pharmacotherapy will precede effective gene therapy, but may well be remarkably effective in preventing/arresting the course of CF lung disease.

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## DISCUSSION

**Brater,** Indianapolis: In your model what does amiloride do?

**Boucher,** Chapel Hill: Amiloride is one of my favorite drugs. Amiloride does block ASL hyperabsorption by CF airway epithelia. The problem with the amiloride is that it was developed as an oral diuretic and so it was designed to be rapidly absorbed. If you apply amiloride to an airway surface by an aerosol, it expands the amount of airway surface liquid greatly and increases mucus clearance, but it does so for only forty minutes. The reason is that the airways epithelia are much like GI epithelia, in that they both express cellular paths that absorb amiloride rapidly. I think that if one had an insight in how to stabilize amiloride or prolong its action on airway surfaces that this would be a great benefit for CF lung disease. The problem is once amiloride is absorbed from the airway lumen, it is no longer effective and is no longer "interesting." So what we need to do is in the inverse of what Merck tried to do 30 years ago with amiloride. It's the same channel, same target, same $IC_{50}$, but we need to make the drug non-absorbable, i.e. "topical."

**Glassock,** Laguma Niguel: What about inhalation of osmotic agents such as polyethylene glycol?

**Boucher:** That's a great question. Osmotic agents work, and the nice thing about using them to treat CF lung disease is that you don't really care which of these two hypotheses are correct as long as the agent is effective. Osmotic agents do expand ASL volume, and they do dilute the ASL sodium chloride concentrations. The problem is we are all "designed" not to inhale large masses of material. So to inhale a sufficient mass of osmotically active material to meaningfully expand the volume of liquid on airway surfaces would require far more material than your upper pharynx and its neural reflexes would allow you to inhale. It is a good idea; the "devil" is in the aerosol delivery "details."